Exp Oncol 2021
43, 2, 118–124

Experimental ONCOLOGY

IN VITRO MODELING OF TUMOR INTERCLONAL INTERACTIONS USING BREAST CANCER CELL LINES

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Background: **In the setting of limited resources, natural selection begins to occur between tumor clones. An experimental model of *in vitro* tumor heterogeneity would allow us to evaluate various types of biological interactions arising from the joint cultivation of phenotypically different tumor clones. *Aim*: To study the peculiarities of ecological relationships of breast cancer (BC) cell lines MCF-7, BT-474 and MDA-MD-231 under co-culturing conditions. *Materials and Methods*: Three BC cell lines: luminal A — MCF-7, luminal B — BT-474 and triple-negative — MDA-MD-231 were co-cultured pairwise. Immunocytochemistry was used to differentiate the cell lines in the wells. The effect of the cell-free culture medium on the growth rate of the alternate cell line in the pair was also evaluated. *Results:* It was shown that when BT-474 cells were co-cultured with MCF-7 and BT-474 cells were co-cultured with MDA-MD-231, two types of ecological interactions could be observed: commensalism and amensalism, respectively. While the cells do not interact with each other in contact, the supernatants of single cultures of MCF-7 and MDA-MD-231 exert the same effect on BT-474 as co-cultivation of BT-474 with these cells. *Conclusions:* The paracrine mechanism of intercellular interaction between different human BC cell lines has been demonstrated. The models used in population ecology can be applicable to identify the types of interaction between cell lines.**

***Key Words*: breast cancer cell lines, cell-cell interaction, cancer ecology, cancer evolution, natural selection.**

DOI: 10.32471/exp-oncology.2312-8852.vol-43-no-2.16142

It is known that tumors are heterogeneous consisting of genetically and phenotypically different malignant clones of tumor cells formed during the clonal evolution of a tumor [1]. Heterogeneity as a result of clonal evolution can occur at the genetic, biochemical, molecular, phenotypic and morphological levels [1–3]. For example, in breast cancer (BC), there is a significant heterogeneity in the expression of estrogen receptor, which determines sensitivity to hormonal therapy in luminal BC type [4].

In the constantly changing conditions of the microenvironment, intratumoral heterogeneity is a consequence of the evolution of malignant cells. The individual genetic features of each clone form a certain landscape, which reflects the overall effectiveness of the tumor adaptation to the evolutionary pressure exerted by tumor host [5]. The high diversity of tumor clones in conditions of limited resources increases the efficiency of natural selection contributing to the emergence of more and more malignant clones. The most interesting consequence of intratumoral heterogeneity is the emergence of various kinds of ecological relationships between tumor clones, both negative and positive, which can affect significantly the course and outcome of the disease.

The negative interactions of tumor clones (predation, parasitism, amensalism) were observed only under experimental conditions, when two tumor cell lines were injected into the animal. In the experiments of Miller *et al.* [6] and Caignard *et al.* [7], upon the inoculation of the mixtures of tumor cell lines to the same animal, the cells of one line inhibited the growth of the cells of another line. Having marked two tumor clones with vital dsRED and GFP fluorescent dyes, Blackburn *et al.* [8] observed the effect of amensalism in the body of Danio fish over time [8]. At the same time, the data for negative types of interactions of tumor clones *in vivo* in humans have not been described probably because the outsider clone is quickly eliminated from the tumor mass due to natural selection.

On the contrary, the positive interactions of tumor clones are described widely, both in experiments on animals and in real clinical setting using multi-color imaging of histological sections of tumors. Baban *et al.* [9] and Tsuji *et al.* [10] observed mutually beneficial cooperation of tumor clones, which initially have different biological properties (high metastatic, but low invasive potential, and *vice versa*). The conclusion about the symbiotic relationship is based on the observation that the joint inoculation of tumor clones in animals produced metastases containing cells with low metastatic potential, which do not form metastases upon single inoculation in the control.

A number of recent papers have presented evidence of the presence of several malignant clones in a tumor differing in their functions. For example, in the study of tumor progression, the presence of a marginal invasive front of a primary tumor is shown, the cells of which due to the secretion of matrix

Submitted: February 19, 2020.
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Abbreviations used: BC – breast cancer; ICC – immunocytochemistry.

metalloproteinases destroy the extracellular matrix. They are followed by the rest of the pool of cells allowing for the escape of tumor cells from the site of the pressure exerted by the immune system facilitating dissemination of tumor cells in the body [11].

The use of modern imaging methods makes it possible to label and examine on a single histological section several areas of the tumor bed, such as an invasive front, center and periphery, as well as to assess the state of the immune environment. Understanding the spatial relationships of various tumor clones with each other and an inflammatory infiltrate, analysis of intercellular interactions and communications, development of mathematical models of tumor cellular composition can reveal new markers for cancer diagnosing and treatment [12]. However, at present, there is no clear understanding of the molecular mechanisms, due to which some tumor clones are maintained or suppressed by others. For this, the first step is the development of an experimental model of *in vitro* tumor heterogeneity, where, under standardized conditions, it would be possible to evaluate different types of biological interactions arising from the joint cultivation of phenotypically different tumor clones.

In this regard, we have studied the model of intratumoral heterogeneity, in which BC cell lines served as the analogues of tumor clones. The intratumoral heterogeneity of BC is well known and described, and there are more than 100 BC cell lines, among which it is easier to choose lines suitable for co-culturing. To assess the interclonal interactions, we selected MCF-7, BT-474 and MDA-MD-231 cell lines isolated from patients with different molecular subtypes (luminal-A, luminal-B and triple negative BC, respectively). This decision was based on three observations. First, different molecular subtypes can be observed in one person [13]. Secondly, tumors with different subtypes have a different prognosis, i.e. have initially different biological behavior [14]. Third, the lines differ in the expression of estrogen, progesterone and HER2 receptors, due to which they can be easily recognized.

The aim of the work was to study the peculiarities of ecological relationships of BC cell lines MCF-7, BT-474 and MDA-MD-231 under co-culturing conditions.

MATERIALS AND METHODS

Cell lines. MCF-7, BT-474 and MDA-MB-231 BC cell lines originated from patients with luminal-A, luminal-B, and triple-negative BC were provided by the Cell Culture Bank of the N.N. Blokhin National Medical Research Centre of Oncology of the Health Ministry of Russia. Their molecular phenotypes were as follows: MCF-7 — ER+/HER2-/PR-; BT-474 — ER+/Her2+/PR-; and MDA-MB-231 — ER-/HER2-/PR-. All the lines were represented by the adhesive cells of epithelial morphology with a doubling time of 50–70 h. The cells were cultured in DMEM with 10% FBS at 37 °C in atmosphere of 5% CO_2.

Design of experiment. The investigated cell lines at the initial concentrations of 10,000 cells/cm^2 (MCF-7 and MDA-MB-231) and 30,000 cells/cm^2 (BT-474) were planted in a 24-well plate. Starting from the second day of incubation, co-incubated cells and control cells (cultured in monoculture) were removed from the wells daily for 7 days. The experiment was performed in triplicate, and at each time point cells counts assessed in Goryaev chamber were averaged. Identification of co-incubated cell lines was carried out using immunocytochemistry (ICC) based on different receptor expression (ER and HER2). In each well with co-incubated cultures, the percentage of cells of a particular cell population was counted.

Immunocytochemical analysis. The mixture of removed cells was transferred onto a glass slide using a Cytospin 4 centrifuge (ThermoFisher Scientific, USA), fixed (fixation 10 min in 2% paraformaldehyde, permeabilization 15 min using 0.5% Triton X-100, then fixing for 10 min in 4% paraformaldehyde), treated with primary antibodies to ER and HER2 (Dako, USA) and secondary antibodies conjugated with horseradish peroxidase (Dako, USA). The staining protocol included peroxidase block (5 min) (Dako, USA), application of primary antibodies (25 min), secondary antibodies (20 min), DAB chromogen (5 min) (Dako, USA), and hematoxylin staining. The number of stained and unstained cells was counted using a microscope supplied with a camera. The total number of counted cells from one transfer was within 6–10 thousand.

Analysis of the spatial arrangement of cells of different cell lines during co-incubation. To study the propagation characteristics of MCF-7, BT-474 and MDA-MB-231 cells under conditions of co-culture, we applied preliminary fluorescent staining of BT-474 with CFSE viable dye (BD Biosciences, USA) before their joint subinoculation according to the manufacturer's protocol. We evaluated the relative position of the studied cultures during co-cultivation on the basis of photographs obtained using a Cytation 3 cell analyzer (Bio-Tek, USA).

Analysis of the role of distant interactions in cellular interaction. To clarify the possible mechanism of cellular interaction, we investigated the effect of cell-free culture medium of one cell line on the growth of another. The medium was obtained in the following way. Cell lines MCF-7, BT-474 and MDA-MB-231 at initial concentration of 10^6 cells were incubated for 4 days in 10 ml of DMEM with 10% FBS, after which the culture medium was collected, purified from cells by centrifugation (1,200 rpm, 3 min), aliquoted and frozen once at -20 °C.

Then, using xCELLegence cell analyzer (ACEA-Biosciences, USA), we studied the effect of each collected culture medium of MCF-7, BT-474 and MDA-MB-231 cell lines on the growth of these cultures in real time. The xCELLigence system uses custom-designed plates with a high-density gold electrode array upon which the target cells adhere and grow. Cells adhere to the plate surface and influence the electrical impedance across the array, which is measured and recorded by the xCELLigence software. The impedance

values are converted by the software into cell index (CI), which is then used as a measure of adhesion.

250 µl of DMEM with 10% FBS and 10,000 cells of the corresponding cell line were added to each well. Further, in each 4 wells, we added 250 µl of saline, culture medium from MCF-7, culture medium from BT-474, or culture medium from MDA-MB-231 and incubated the cells up to 300 h. The graphs obtained reflected the increase in cell mass in each cell during incubation.

Statistical analysis. The statistical significance of differences in the number of cells in the wells of the plate was evaluated by the non-parametric Mann — Whitney test.

RESULTS

For reliable subsequent differentiation of co-cultured cell lines by ICC, we evaluated the staining of HER2 and ER cell receptors in the original cultures. It turned out that the surface-expressed HER2 receptor in BT-474 culture was stained stably, while the nuclear ER staining in MCF-7 and BT-474 was weak and varied greatly from cell to cell (Fig. 1). In this regard, we decided to further evaluate only two combinations of cell cultures, which we could confidently distinguish by HER2 expression: MCF-7/BT-474 and BT-474/MDA-MB-231.

On days 2 through 7, we estimated the growth of cells of each culture under conditions of co-cultivation and compared them with cultures of individual lines as controls. The presence of MCF-7 cells in the co-culture contributed to the increase in the number of BT-474 cells compared to their control values, whereas the number of MCF-7 cells themselves was at the same level as in the control fluctuating within

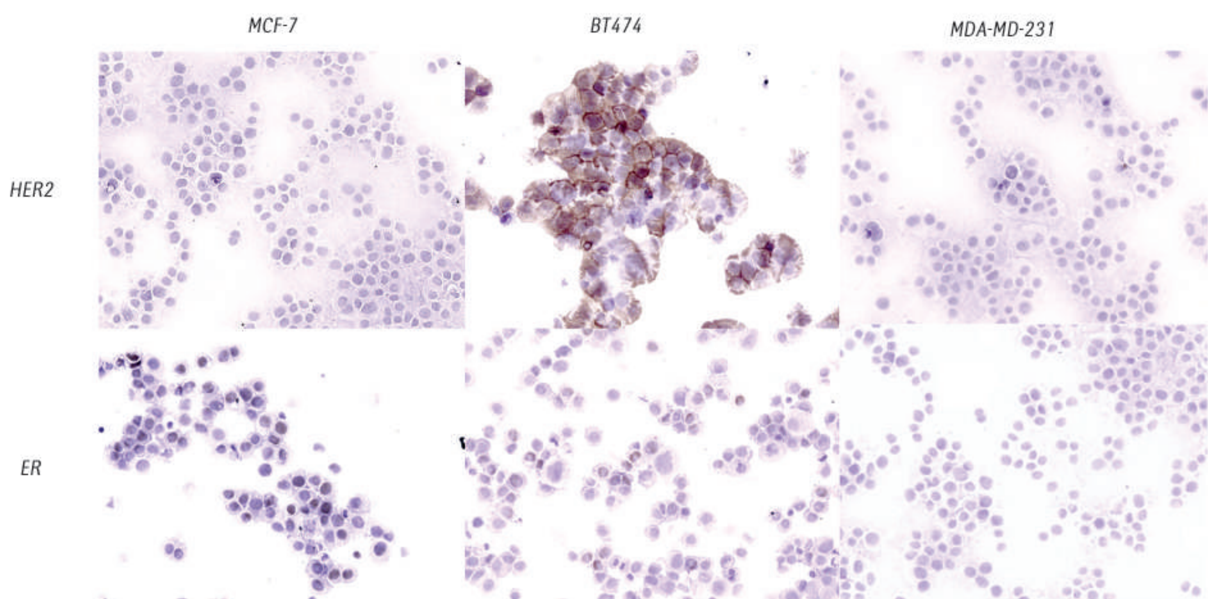

Fig. 1. ICC staining of HER2 and ER receptors in MCF-7, BT-474 and MDA-MB-231 cells, × 200

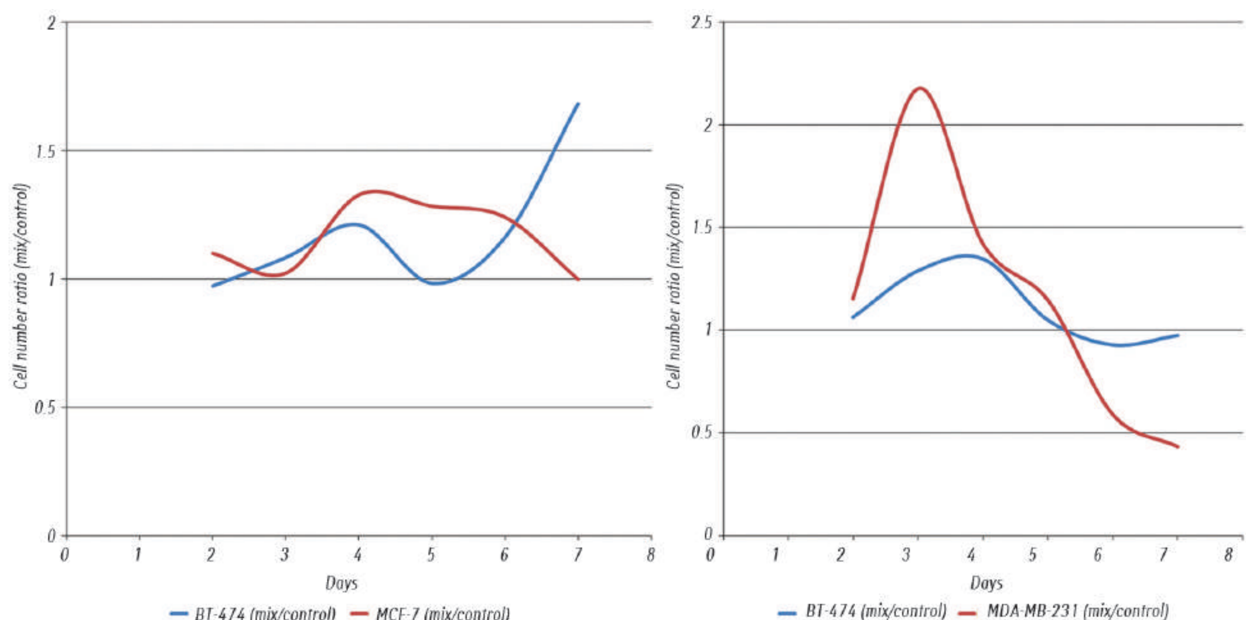

Fig. 2 The ratio of cell number during co-cultivation (mix) to cell number of the same line in the control for the BT-474/MDA-MB-231 and BT-474/MCF-7 pairs

20% (Fig. 2). A statistically significant increase in the number of BT-474 cells in a co-culture compared with the control was observed only on day 7 ($p < 0.05$) probably due to the low growth rate of BT-474. This type of positive relationship, when one cell line benefits from co-existence with another one without harming could be considered as commensalism.

When co-cultivating MDA-MB-231 and BT-474 cells, on the contrary, a negative type of interaction was observed: BT-474 cells inhibited the growth of MDA-MB-231 cells, while the number of BT-474 cells did not change in comparison with the control. On day 2, the number of MDA-MB-231 cells during co-culture was higher than in the control, but by day 7, their number was lower than in the control ($p < 0.05$). According to the ecological interpretation, this type of relationship, in which one cell line undergoes inhibition of growth and development, and the second cell line is not subjected to such changes could be considered as amensalism (see Fig. 2).

To assess whether there are any contacts between cells of different lines when they are co-cultured, BT-474 cells were previously stained with the vital CFSE dye (Fig. 3).

According to the photographs, the cells were evenly spaced along the surface of the well; no peculiar features were found. The BT-474 cell line (marked with green) is characterized by the presence of a large number of intercellular contacts with the cells of its line, so they grow as a dense group of cells. MCF-7 or MDA-MB-231 cell lines are evenly distributed around them (cells are not stained, only the nuclei are visible). Close cell-to-cell contacts of cells from different cultures were not observed at different periods of dynamic observation, and in this connection the assumption about the contact-mediated type of interaction in the 2D model conditions was not confirmed.

To test the assumption of the paracrine mechanism of the influence of one cell culture on another, we evaluated the change in the growth dynamics of one cell line when adding a cell-free culture medium to it, in which another line was incubated. The proliferative activity of cells was monitored in real time using the iCELLegence cell analyzer (ACEABiosciences, USA).

It was found that the addition of medium from MCF-7 accelerates the growth of BT-474 compared to the control (Fig. 4), while the addition of medium from BT-474 inhibits the growth of MDA-MB-231 (Fig. 5), which corresponds to the results obtained using ICC after co-cultivation of tumor cell lines (see Fig. 2).

DISCUSSION

Modeling the processes of cell interactions occurring in tumors *in vivo* is a very difficult task, since the state of intratumoral heterogeneity is largely determined by tumor microenvironment. According to the literature data, the extracellular matrix, the vasculature and the immune cell infiltrate have the greatest impact on the diversity of tumor cells [15]. A different combination of these factors provides selective pressure on tumor cells leading to increased intratumoral heterogeneity arising in the process of clonal evolution [16]. With three-dimensional models of BC cell lines, their great tumorigenicity is shown. Matrigel or fibroblasts enhance tumorigenicity of BT-474, MCF-7, T-47D, MDA-MB-231 cells inoculated to the nude mice [17, 18]. In this regard, the simulation of interclonal interactions in 2D-culture is a significant simplification of the real situation but enables us to study the molecular changes in different types

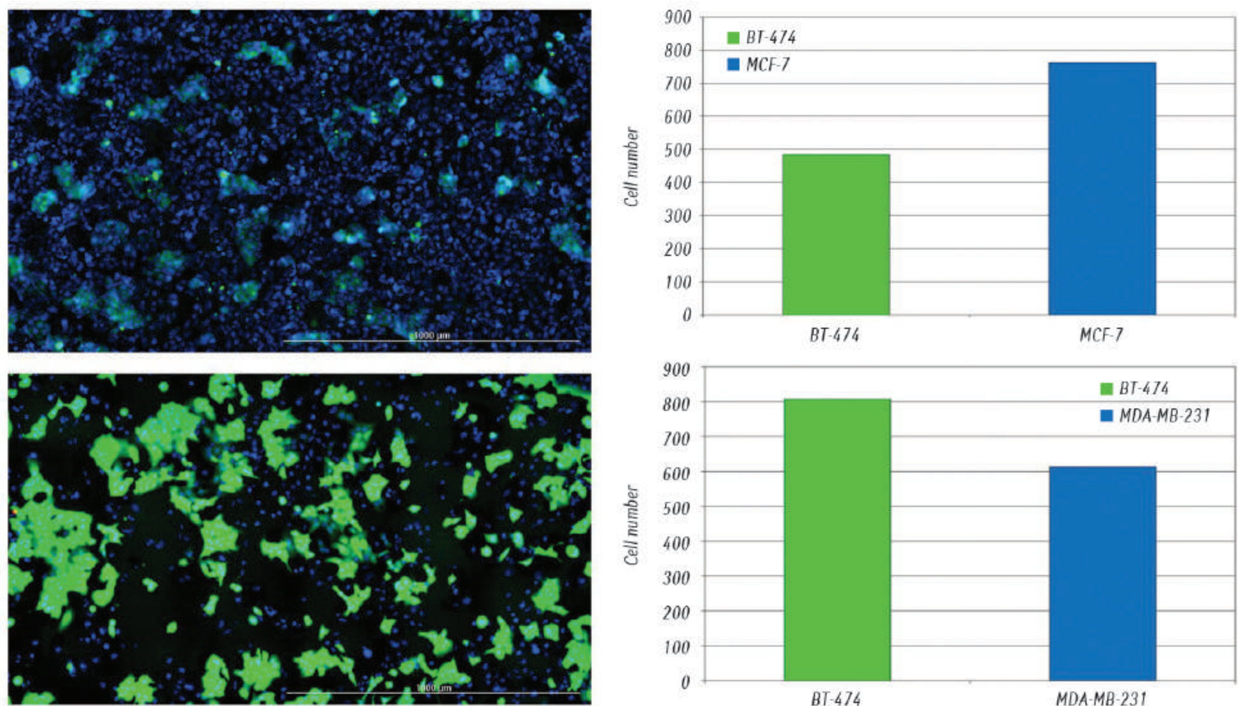

Fig. 3 Micrographs and the total number of vital-stained BT-474 cells under co-culture with MCF-7 and MDA-MB-231 cells

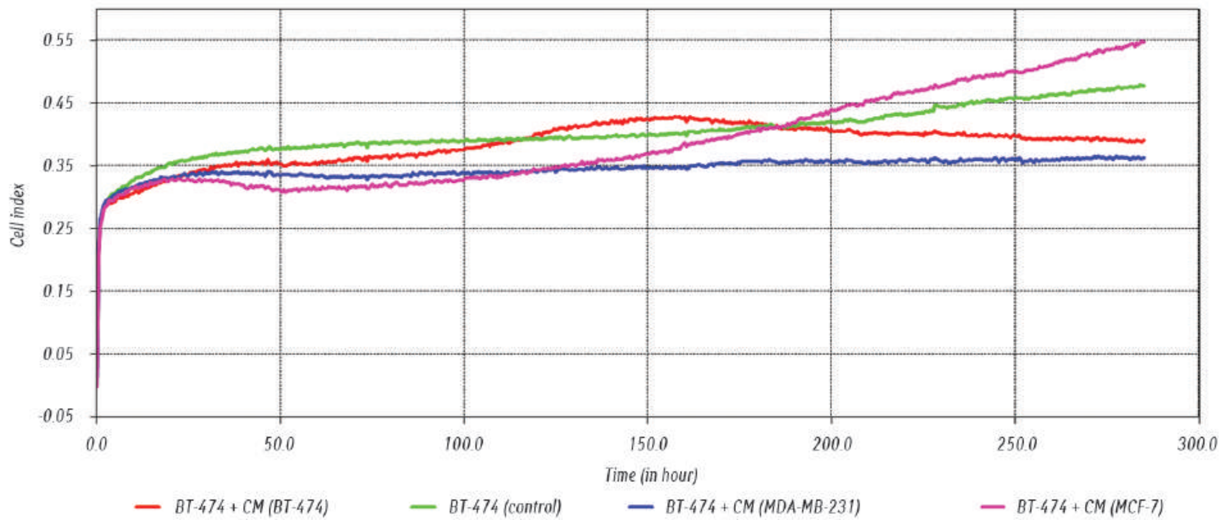

Fig. 4 Growth dynamics of BT-474 cells upon the addition of supernatants from MDA-MB-231, MCF-7 and BT-474 cell cultures

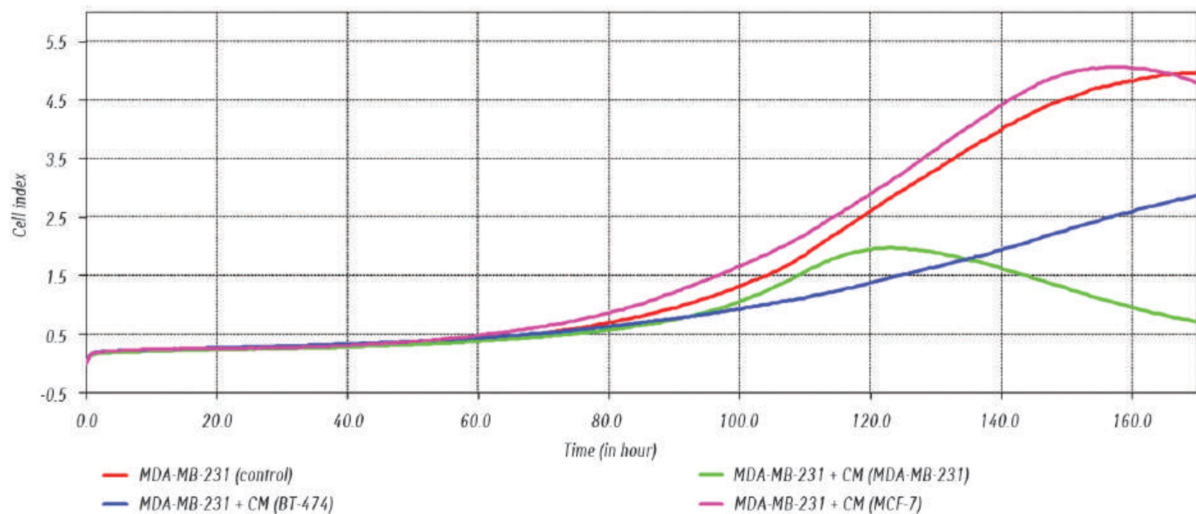

Fig. 5. Growth dynamics of MDA-MB-231 cells upon the addition of supernatants from MDA-MB-231, MCF-7 and BT-474 cell cultures

of interactions, caused exclusively by the influence of cells of another clone.

We have demonstrated the positive or negative cell-cell interactions between BT-474 cell line and co-cultured cells, namely commensalism in a case of MCF-7 cells and amensalism in a case of MDA-MB-231 cells.

Two mechanisms of cellular interactions are possible — distant and contact-mediated [19, 20]. Contact-mediated influence suggests juxtacrine signaling in cell-cell contacts via ligand-receptor interaction, for example, Notch signaling cascade [21], thin membrane cytoplasmic connections — tunneling nanotubes [22], gap junctions [23], desmosomes [24], etc. Distant signal transmission is possible through paracrine secretion of various biological substances (cytokines, growth factors, microRNAs, extravesicles) to the environment [25, 26]. To assess the mechanisms involved in this experiment we evaluated the mutual spatial arrangement of cells of different lines during their joint passage, and also evaluated the effect of secreted factors of one culture on the reproduction rate of another one.

We did not reveal close contacts between co-cultured cells of BT-474/MCF-7 and BT-474/MDA-MB-231 lines suggesting that the contact-mediated type of interaction is unlikely. On the contrary, the influence of the culture medium of one cell line on the growth rate of another one evidences on the distant type of interaction.

According to the literature data, MCF-7, BT-474 and MDA-MB-231 cell lines are characterized by an individual secretory profile that differs in the composition of cytokines, chemokines, growth factors, collagenases, exosomes, etc. MCF-7 cell line has an increased production of IL-1β, IL-2, IL-12, IGF-1, TNF-α; BT-474 cell line — IL-10, CRAM-B, MMP-16, E-cadherin; and MDA-MB-231 cell line — IL-8, IL-10, ADAM17, PIAUR, ITGA6, MMP-3, CXCL1, CCL-18, VEGF [27–31].

Hanahan *et al.* [32] described 6 major signs of cancer, among which the self-sufficiency of tumor cells in relation to growth factors was mentioned. Probably, in the early stages of carcinogenesis, tumor cells passively adapt to the secretory microenvironment, but later, under the influence of natural selection, they acquire the ability to independently supply themselves with growth factors, forming for themselves the conditions in which their survival rate will be maximal.

One of the possible ways of providing the necessary signaling environment consists in modulating cells of the immune system. Polarization of the immune response along the Th2 pathway increases the risk of tumor progression, which is associated with the formation of IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, TGF-β and other cytokines [33]. It has been shown that tumor cells induce M2 polarization of macrophages secreting IL-10 and TGF-β, VEGF into the environment, which also leads to tumor progression and significantly reduces the overall and relapse-free survival of patients with BC [34]. Also in the *in vitro* model, a positive feedback mechanism is shown, when the MDA-MB-231 cells capable of producing IL-8 stimulate the formation of the same interleukin by fibroblasts/macrophages in the case of co-incubation, which enhances the proliferation and migration of tumor cells [35].

The second possible way to create a suitable secretory environment for tumor cells is to acquire the ability to independently synthesize the necessary growth factors (auto and paracrine self-regulation). ER^-/PR^- cells express both a series of growth factors (i.e., EGF, TGF-alpha, amphiregulin, heregulin, FGFs, IGFs) and their corresponding receptors more frequently than ER^+ and/or PR^+ BC cells, thus sustaining growth independently of exogenous growth factor supply (autocrine loop) [36].

BC cell lines isolated from different patients and having a different set of mutations have different protein expression profiles, microRNA, microvesicles [26, 37, 38], many of which affect cell proliferation. Given that almost all tumors are heterogeneous and consist of different cell populations differing in phenotypic and genotypic traits for tumor growth, it is sufficient that at least a small part of tumor cells provides the necessary growth factors. From the point of view of evolutionary biology, in this case a positive type of interaction (mutualism /commensalism) is formed between the tumor cells, as a result of which their overall fitness increases. Destroying the existing ecological system of tumor cells and directing them towards negative interactions, we can significantly advance in the search for new approaches to treatment of malignant tumors.

ACKNOWLEDGMENTS

The reported study was funded by RFBR, project number 18-29-09131 “The Phenomenon of Tumor “Fading” During Chemotherapy”.

REFERENCES

1. **Ibragimova M, Tsyganov M, Litviakov N.** Natural and chemotherapy-induced clonal evolution of tumors. Biochemistry (Moscow) 2017; **82**: 413–25.

2. **Janiszewska M, Polyak K**. Clonal evolution in cancer: a tale of twisted twines. Cell Stem Cell 2015; **16**: 11–2.

3. **Dagogo-Jack I, Shaw AT** Tumour heterogeneity and resistance to cancer therapies. Nat Rev Clin Oncol 2018; **15**: 81–94.

4. **Hammond MEH, Hayes DF, Dowsett M, *et al.*** American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer (unabridged version). Arch Pathol Lab Med 2010; **134**: e48–72.

5. **Merlo LM, Pepper JW, Reid BJ, Maley CC.** Cancer as an evolutionary and ecological process. Nat Rev Cancer 2006; **6**: 924–35.

6. **Miller BE, Miller FR, Wilburn D, *et al.*** Dominance of a tumor subpopulation line in mixed heterogeneous mouse mammary tumors. Cancer Res 1988; **48**: 5747–53.

7. **Caignard A, Martin M, Michel M, *et al.*** Interaction between two cellular subpopulations of a rat colonic carcinoma when inoculated to the syngeneic host. Int J Cancer 1985; **36**: 273–9.

8. **Blackburn JS, Liu S, Wilder JL, *et al.*** Clonal evolution enhances leukemia-propagating cell frequency in T cell acute lymphoblastic leukemia through Akt/mTORC1 pathway activation. Cancer Cell 2014; **25**: 366–78.

9. **Baban D, Matsumura Y, Kocialkowski S, Tarin D.** Studies on relationships between metastatic and non-metastatic tumor cell populations using lineages labeled with dominant selectable genetic markers. Int J Dev Biol 2003; **37**: 237–43.

10. **Tsuji T, Ibaragi S, Shima K, *et al.*** Epithelial-mesenchymal transition induced by growth suppressor p12CDK2-AP1 promotes tumor cell local invasion but suppresses distant colony growth. Cancer Res 2008; **68**: 10377–86.

11. **Turunen SP, Tatti-Bugaeva O, Lehti K.** Membrane-type matrix metalloproteases as diverse effectors of cancer progression. Biochim Biophys Acta Mol Cell Res 2017; **1864**: 1974–88.

12. **Giesen C, Wang HA, Schapiro D, *et al.*** Highly multiplexed imaging of tumor tissues with subcellular resolution by mass cytometry. Nat Meth 2014; **11**: 417–22.

13. **Davila E, Amazon K.** The clinical importance of the heterogeneity of HER2 neu. Case Rep Oncol 2010; **3**: 268–71.

14. **Carey LA, Perou CM, Livasy CA, *et al.*** Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA 2006; **295:** 2492–502.

15. **Scheele CL, Maynard C, van Rheenen J.** Intravital insights into heterogeneity, metastasis, and therapy responses. Trends Cancer 2016; **2**: 205–16.

16. **Bonnans C, Chou J, Werb Z.** Remodelling the extracellular matrix in development and disease. Nat Rev Mol Cell Biol 2014; **15**: 786–801.

17. **Van Slooten H, Bonsing B, Hiller A, *et al.*** Outgrowth of BT-474 human breast cancer cells in immune-deficient mice: a new *in vivo* model for hormone-dependent breast cancer. Br J Cancer 1995; **72**: 22–30.

18. **Mullen P, Ritchie A, Langdon SP, *et al.*** Effect of Matrigel on the tumorigenicity of human breast and ovarian carcinoma cell lines. Int J Cancer 1996**; 67:** 816–20**.**

19. **Mattes B, Scholpp S.** Emerging role of contact-mediated cell communication in tissue development and diseases. Histochem Cell Biol 2018; **150:** 431–42.

20. **Nishida-Aoki N, Gujral TS.** Emerging approaches to study cell–cell interactions in tumor microenvironment. Oncotarget 2019; **10**: 785–97.

21. **Fortini ME.** Notch signaling: the core pathway and its posttranslational regulation. Dev Cell 2009; **16**: 633–47.

22. **Gerdes H-H, Carvalho RN.** Intercellular transfer mediated by tunneling nanotubes. Cur Opin Cell Biol 2008; **20**: 470–5.

23. **Sinyuk M, Mulkearns-Hubert EE, Reizes O, *et al.*** Cancer connectors: Connexins, gap junctions, and communication. Front Oncol 2018; **8:** 646.

24. **Bhat AA, Uppada S, Achkar IW, *et al.*** Tight junction proteins and signaling pathways in cancer and inflammation: a functional crosstalk. Front Physiol 2019; **9:** 1942.

25. **Davies AE, Albeck JG.** Microenvironmental signals and biochemical information processing: cooperative determinants

of intratumoral plasticity and heterogeneity. Front Cell Dev Biol 2018; **6:** 44.

26. **Li I, Nabet BY.** Exosomes in the tumor microenvironment as mediators of cancer therapy resistance. Mol Cancer 2019; **18**: 32.

27. **Joimel U, Gest C, Soria J, *et al*.** Stimulation of angiogenesis resulting from cooperation between macrophages and MDA-MB-231 breast cancer cells: proposed molecular mechanism and effect of tetrathiomolybdate. BMC Cancer 2010; **10**: 375.

28. **Chang G, Wang J, Zhang H, *et al*.** CD44 targets Na^+/H^+ exchanger 1 to mediate MDA-MB-231 cells' metastasis via the regulation of ERK1/2. Br J Cancer 2014; **110:** 916–27.

29. **Bottai G, Raschioni C, Székely B, *et al*.** AXL-associated tumor inflammation as a poor prognostic signature in chemotherapy-treated triple-negative breast cancer patients. NPJ Breast Cancer 2016; **2**: 1–10.

30. **Escobar P, Bouclier C, Serret J, *et al*.** IL-1β produced by aggressive breast cancer cells is one of the factors that dictate their interactions with mesenchymal stem cells through chemokine production. Oncotarget 2015; **6**: 29034.

31. **Su S, Liu Q, Chen J, *et al*.** A positive feedback loop between mesenchymal-like cancer cells and macrophages is essential to breast cancer metastasis. Cancer Cell 2014; **25**: 605–20.

32. **Hanahan D, Weinberg RA.** The hallmarks of cancer. Cell 2000; **100**: 57–70.

33. **Joyce JA, Fearon DT.** T cell exclusion, immune privilege, and the tumor microenvironment. Science 2015; **348**: 74–80.

34. **Yang M, McKay D, Pollard JW, et al.** Diverse functions of macrophages in different tumor microenvironments. Cancer Res 2018; **78**: 5492–503.

35. **Jin K, Pandey NB, Popel AS.** Crosstalk between stromal components and tumor cells of TNBC via secreted factors enhances tumor growth and metastasis. Oncotarget 2017; **8**: 60210.

36. **Ethier SP.** Growth factor synthesis and human breast cancer progression. J Natl Cancer Inst 1995; **87**: 964–73.

37. **Aharon A, Sabbah AR, Issman L, *et al*.** Effects of low- and high-dose chemotherapy agents on thrombogenic properties of extracellular vesicles derived from breast cancer cell lines. Thromb Haemost 2018; **118**: 480–9.

38. **Shi Y, Ye P, Long X.** Differential expression profiles of the transcriptome in breast cancer cell lines revealed by next generation sequencing. Cell Physiol Biochem 2017; **44**: 804–16.

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МОДЕЛЮВАННЯ *IN VITRO* ВЗАЄМОДІЇ МІЖ ПУХЛИННИМИ КЛОНАМИ КЛІТИННИХ ЛІНІЙ РАКУ ГРУДНОЇ ЗАЛОЗИ

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Стан питання: За умов обмежених ресурсів починає діяти природний відбір між клонами пухлинних клітин. Експериментальна модель гетерогенності пухлин *in vitro* дозволяє оцінити різні типи біологічної взаємодії при сумісному культивуванні фенотипові різних клонів пухлинних клітин. ***Мета*:** Дослідити особливості екологічних взаємовідносин між клітинними лініями раку грудної залози MCF-7, BT-474 та MDA-MD-231 при їх сумісному культивуванні. ***Матеріали та методи*:** Попарно сумісно культивували наступні три лінії клітин раку грудної залози MCF-7 (люмінальний рак підтипу А), BT-474 (люмінальний рак підтипу В) та MDA-MD-231 (тричі негативний рак). Клітини різних ліній у чарунках планшетів диференціювали за допомогою імуноцитохімічного забарвлення. Вивчали також ефекти безклітинного культурального середовища на ріст окремо взятих клітин кожної з взаємодіючих пар. ***Результати:*** У разі сумісного культивування клітин BT-474 з клітинами MCF-7, а також сумісного культивування клітин BT-474 з клітинами MDA-MD-231 спостерігали два типи екологічних взаємовідносин: коменсалізм та аменсалізм відповідно. Контактної взаємодії між клітинами різних ліній відзначено не було. Водночас безклітинне культуральне середовище клітин MCF-7 та MDA-MD-231 справляло такий самий ефект на ріст клітин BT-474, що й при сумісному культивуванні. ***Висновки:*** Продемонстровано, що взаємодія між клітинами різних ліній раку грудної залози людини відбувається за паракринним механізмом. Моделі, які використовуються в популяційній екології, можуть застосовуватися для ідентифікації типів взаємодії між різними лініями клітин.

***Ключові слова*:** лінії клітин раку грудної залози, міжклітинні взаємодії, екологія популяцій пухлинних клітин, природний відбір.